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IN VITRO GLUCOSE REVERSAL OF THE INHIBITORY EFFECT OF FASTING ON EPINEPHRINE-INDUCED LIPOLYSIS.

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SUMMARY:

Cyclic AMP, protein kinase activity and glycerol were measured in adipose tissue from fasted rats incubated with epinephrine with or without glucose. A drastic loss in the sensitivity of the adipose tissue to respond to the lipolytic action of the hormone was observed during fasting, when incubated without glucose. The addition of glucose reverses this process, and a greater lipolytic capacity was observed in the tissue of fasted rats than in fed rats. The three parameters measured were well correlated when there was epinephrine in the medium. Lipolysis is observed with glucose alone, but there was no variation in the cAMP levels nor in the protein kinase activity. These results are discussed in relation to the regulator effect of FFA, which is mobilized during starvation, on lipolysis.

INTRODUCTION

In vitro studies have well established the importance of the adenyl cyclase-protein kinase system in the hormonal activation of lipolysis in adipose tissue (1,2). However, the mechanisms involved in the stimulating role of glucose (3) or the inhibiting role of free fatty acid (FFA)(4) in lipolysis are still under discusion (5,6). Angel et al.(7) have shown that the intracellular accumulation of FFA produces a decrease in the epinephrine-stimulated lipolysis and the ATP levels. This has been observed by others (8,9). The stability of the FFA inhibiting effect through various treatments is deduced by the interesting observation that adenyl cyclase, in membranes prepared from human adipocytes previously preincubated with FFA, loses its capacity to respond to the epinephrine (9). An inhibition has also been demonstrated in epinephrine-stimulated adenyl cyclase activity of adipocyte plasma membranes from rats fed high fat (10). Fasting produces an increase of lipolysis with a rapid mobilization of FFA (11). Therefore, fasting can

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serve as an experimental model to investigate the effect that high concentrations of plasmatic FFA, maintained for a long time, could exert on the lipolytic sensitivity of adipose tissue. This study examines this possibility, as well as the in vitro effect of glucose on the levels of cyclic AMP, glycerol and protein kinase activity.

MATERIAL AND METHODS

Male Wistar rats weighing 180-200 g were fed on a standard diet or subjected to 24,48 and 96 hours of fasting. They were anaesthetized with Nembutal. The epididymal adipose tissue was divided into small pieces (10-20mg) and randomized so that each flask contained 80-100mg of tissue. The fat pads were incubated and homogenized according to the methods described (12), with 3 mg/ml of bovine serum albumin. The homogenate was centrifuged 15.000 x g for 10 min at 4°C. Aliquots of infranatant were immediately assayed for protein kinase activity (12). The cyclic AMP was measured by the method of Gilman (13), as described (14), in other aliquots previously deproteinized with 10% TCA. The glycerol in the medium was measured by the method described by Garland et al.(15). Protein concentration was estimated by the method of Lowry et al.(16). (γ-^{32}P)ATP and (^{3}H)cyclic AMP were supplied by the Radiochemical Centre, Amersham. Cyclic AMP and other enzymes were obtained from Boehringer, Mannheim, and Histone (type II-A) and epinephrine from Sigma. The protein kinase and its inhibitor were prepared according to Walsh (17) and Gilman (3), respectively.

RESULTS

Cyclic AMP, protein kinase activity and glycerol in fat-pads. Epididymal fat-pads from fed and fasting rats were incubated with (Fig.2) or without (Fig.1) 5 mM glucose, in the presence or absence of 10 μM epinephrine. Fig.1 shows that 10 μM epinephrine activated the synthesis of cAMP in the adipose tissue of fed rats, values that were approximately two times those obtained without the hormone (90 pmol cAMP/mg protein) being reached. This confirms the classical observation that epinephrine stimulates the adenyl cyclase activity from adipose tissue (1). However, there was no change observed in the intracellular concentration of cyclic AMP in tissue from rats fasted during 24, 48 and 96 hours, when incubated with epinephrine. The results obtained were the same as those without epinephrine. An identical pattern was found in the protein kinase activity. There were no significant differences in the cyclic AMP levels nor in the protein kinase activity in the different fasting periods. The lipolytic activity (expressed as glycerol released into the medium) was studied under the same conditions of incubation. The fasting was

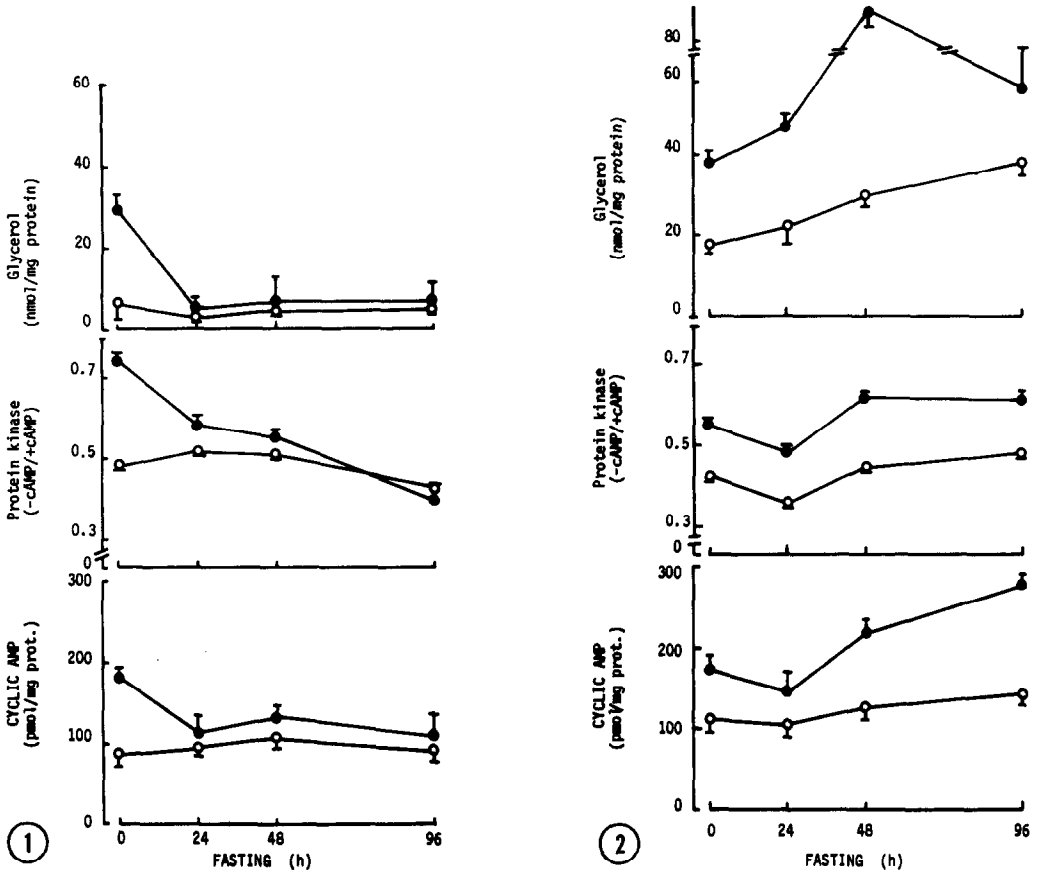

Fig. 1. Effect of epinephrine on cyclic AMP levels, protein kinase activity and glycerol release on different days of fasting.
Pieces of epidydimal adipose tissue were incubated in Krebs Ringer phosphate pH 7.4 for 10 min as described in the text in the presence (●) or absence (○) of 10 μM epinephrine. Each point represents the mean ± SEM of 4 determinations.

Fig. 2. Effect of epinephrine in the presence of glucose on cyclic AMP, protein kinase activity and glycerol release on different days of fasting.
Experimental conditions were the same as those of Fig.1, except that there were 5 mM glucose in the medium of incubation. (●) glucose and epinephrine; (○) Glucose.

associated with a total loss of epinephrine-stimulated lipolysis in adipose tissue incubated without glucose. There was a definite lipolytic activity in rats fed normally, going from 9 nmol(control) to 30 nmol/mg protein of glycerol liberated in the presence of the hormone. It should be pointed out that three parameters studied appear well correlated both in their increment (fed rats) and in their invariability (fasting rats). When fat-pads were

incubated with 5 mM glucose (Fig.2) a different response was observed from the one indicated above. Glucose, by itself, did not produce any variation in the cyclic AMP levels in any feeding stage nor did it change the cyclic AMP basal concentration, 100 nmol/mg protein, in relation to the adipose tissue incubated in its absence. However, epinephrine stimulated the production of cyclic AMP in all the feeding stages. It is also apparent that at 48 hours of fasting the adenyl cyclase-stimulated epinephrine is significantly greater ($p<0.05$, student's t test) in relation to tissue obtained from fed rats. The alterations in the protein kinase activity followed a pattern that was practically the same as that observed for the cyclic AMP. The epinephrine-stimulated lipolysis was increased in the adipose tissue of fasting rats as compared to fed rats. In the absence of epinephrine, glucose stimulated the liberation of glycerol in all the stages of nutrition, this effect being more pronounced as the fasting time was increased.

DISCUSSION

The present study shows a loss in vitro in the sensitivity of the adipose tissue from fasted rats, incubated without glucose, to respond to the epinephrine lipolytic action. The addition of glucose reverses this situation, the lipolytic response to epinephrine being significantly greater in fasting rats than in fed rats. Zapf et al.(18) previously found a reinforcing effect of fasting in the lipolytic effect of epinephrine in adipocytes of fasted rats. The present work, however, presents evidence that such a situation is produced only when glucose exists in the medium. The decreased sensitivity in the adipose tissue from fasted rats towards epinephrine in absence of glucose could be a consequence of different factors. During fasting there have been no modifications described in the intracellular concentration of FFA in adipose tissue (19). However, their concentration in the extravascular-extracellular space surrounding the adipocytes could be conceivably much higher (9). It has also been well established that the intracellular accumulation of FFA leads to a decrease in lipolysis (6,7,9) due to an

inhibition of the adenyl cyclase (20) or of the oxidative phosphorylation (21). In the data presented (Fig.1) no activation of the intracellular cyclic AMP levels in presence of epinephrine is observed. It follows, therefore, that one of the principal reduction mechanisms of the lipolysis consists of the inhibition of the adenyl cyclase activity, similar to that previously described (6,9). The inhibition by fasting of the epinephrine lipolytic action was overcome by the addition of glucose to the incubation medium. Glucose alone, in rats fed normally, stimulates the liberation of glycerol in the medium to almost two times the levels of the controls, this effect being more pronounced in the adipose tissue of fasted rats. This lipolytic effect of the glucose has been observed by others (4,22,23) although negative results have also been described (24). Desai et al.(25) have demonstrated that the addition of glucose increases the reesterification of fatty acids, manifested by a decrease in the FFA glycerol ratio. The effect of glucose in the recovery of the lipolytic capacity of the adipose tissue in fasting could be explained as a protective effect of the sugar opposed to the inhibitive role of the FFA, achieved by the stimulation of their reesterification. However, other reported works question this interpretation (5,24,26). From the data of this study, it appears clear that the inhibition or stimulation of the lipolysis on different days of fasting is determined by the intracellular cyclic AMP concentration, indicating a causal relation presumably related to the inhibitive effect of the FFA on the adenyl cyclase, that is modified in presence of glucose. However, the lipolytic effect of glucose alone does not agree with this interpretation. Its action appears to be more manifest as fasting progresses, but without a correlating increase of intracellular cyclic AMP. Knight et al.(5) find that glucose inhibited the fall in the rate of glycerol release but without change in the cyclic AMP levels. The mechanism of glucose control proposed by these authors would be to inhibit the lipase-inactivating system, this mechanism being independent of that mediated by the FFA. Likewise it has been proposed that the increase in

lipolysis from fasted rats is due to an inhibition of the same enzymatic system (27).

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